

In the specification:

After the title, but before the Claim for Foreign Priority, please insert the following new paragraph:

Cross-Reference to Sequence Listing

This application includes as part of the disclosure a sequence listing in CD-ROM format having the file name 1974005-SEQ-ENG, created on April 9, 2003 and comprising 2.54 MB, the contents of which are incorporated by reference in their entirety into the present application.

Please replace paragraph [0021] with the following new paragraph:

[0021] In this context, in the sense of the invention, one understands the statement that “the cell exhibits the necessary genes and/or enzymes for the synthesis of an orthosomycin basic body” to mean that the genes that code for the necessary enzymes and/or the functional enzymes themselves, which are necessary for the synthesis of an “orthosomycin basic body” from the precursor stages that are usually present are present in the cell. Examples would be the gene cluster according to the invention in accordance with Fig. 1 or the “Open Reading Frames” (ORF), and/or genes in accordance with consecutive number 1-54 in accordance with Table 1 in combination with ~~Fig. 1~~ SEQ ID NOS. 1-116, and/or the related enzymes and/or proteins in accordance with consecutive number 55-108 in accordance with Table 1 in combination with ~~Fig. 1~~ SEQ ID NOS. 1-116.

Please replace paragraph [0035] with the following paragraph:

[0035] It is preferred for the production method by way of which the avilamycin derivative according to the invention is defined if the cell that can be cultivated is selected from a cell of the type *Streptomyces viridochromogenes* or a cell that, with the exception of the nucleic acid(s) modified by gene technology, deleted, or not expressed, contains the nucleic acids in accordance with consecutive number 1-54 in accordance with Table 1 in

combination with ~~Fig. 1~~ SEQ ID NOS. 1-116, and/or nucleic acids that are homologous to it by at least 95%, preferably 97%, or hybridizes with one of these sequences under moderately stringent conditions, or contains the gene cluster in accordance with Fig. ~~109~~ 1. The second point of selection is particularly understood to mean cells in which the enzymes necessary for avilamycin derivative synthesis are expressed using gene technology methods, where one of the nucleic acids that codes for the enzyme that occurs endogenously in *Streptomyces viridochromogenes* Tu 57 is modified by gene technology or deleted or not expressed, in particular that the nucleic acid/DNA is not introduced into the host cell by gene technology in the first place. However, it is especially preferred if the cell is selected from a cell of the type *Streptomyces viridochromogenes*, particularly a cell of the type *Streptomyces viridochromogenes* Tu 57 or A 23575.

Please replace paragraph [0040] with the following paragraph:

[0040] Analogously, it is a particularly preferred object of the invention if, with regard to the production process indicated above, the sequence(s) of the modified nucleic acid(s) before being modified correspond(s) by at least 95%, preferably 97%, and particularly precisely to the nucleic acid sequence(s) of at least one of the sequences in accordance with consecutive number 1 or 2-7 in accordance with Table 1 in combination with ~~Fig. 1~~ SEQ ID NOS. 1-116, preferably one of the sequences with consecutive number 1, 2, 4, or 6 (Table 1 in combination with ~~Fig. 1~~ SEQ ID NOS. 1-116), particularly the sequence with consecutive number 2 or the sequences with consecutive numbers 2 and 1, numbers 2 and 4, or numbers 2 and 6 (in accordance with Table 1 in combination with ~~Fig. 1~~ SEQ ID NOS. 1-116, or if it hybridizes with one of these sequences under moderately stringent conditions.

Please replace paragraphs [0045] through [0050] with the following paragraphs:

[0045] It was also a task of the invention – in addition to making new antibiotics available-to clarify the synthesis of avilamycin A, in order to develop new antimicrobial substances based on it, and new methods for their production. A key point in this context was molecular cloning and characterization of the genes involved in avilamycin biosynthesis.

A piece with a size of about 60kb around the known genes *avid*, *aviE1*, and *aviM* was sequenced. In this context, it turned out that the genes involved were arranged in the immediate vicinity of one another in a cluster. The sequence of the individual ORFs as well as their arrangement on the central gene cluster (consecutive numbers 1 to 54) are ~~shown in Fig. 1~~ given in SEQ ID NOS. 11 to 116 in combination with Table 1 as in Fig. 109 ~~1~~. As already explained, the sequence of an NDP-glucose-synthase gene (*aviD* [consecutive number 53 in accordance with Table 1 in combination with ~~Fig. 1~~ SEQ ID NO.: 53]), and NDP-glucose-4,6-dehydratase gene (*aviE1* [consecutive number 54 in accordance with Table 1 in combination with ~~Fig. 1~~ SEQ ID NO.: 55]), and a polyketide synthase gene (*aviM* [consecutive number 52 in accordance with Table 1 in combination with ~~Fig. 1~~ SEQ ID NO.: 51]) were known, as was their presumed function as part of an interactive Type I polyketide synthase for the formation of orsellinic acid, an intermediate product in the biosynthesis of dichoroisoeverminic acid [Gaisser, S., Trefzer, A., Stockert, S., Kirschning, A., and Bechthold, A., *J. Bacteriol.*, 179:6271-6278 (1997)].

[0046] The sequences of the other ORFs involved in the synthesis of avilamycins, which were discovered as a result of extensive cloning, are given in SEQ ID NOS.: 11-116; their relative arrangement on the gene cluster is shown in Fig. 1. Here, giving the consecutive number from Table 1 makes it possible to assign the designation of the ORFs by name. Under the designation by name, the sequences can be derived from ~~Fig. 1~~ SEQ ID NOS.: 11-116. The precise cloning strategy as well as further details regarding sequencing are presented in the examples, as are the functional analysis and characterization of the genes found (ORFs). The assignment of ORF abbreviations to function and sequence (including derived protein sequence) can be derived from Table 1, which follows the figure description.

[0047] Another important object of the invention is therefore one (or several) nucleic acid(s) that correspond(s) by at least 95%, preferably 97%, and particularly precisely to the nucleic acid sequence in accordance with one of the sequences of consecutive number 1 to 51 in accordance with Table 1 ~~in combination with Fig. 1~~ in combination with SEQ ID NOS.: 11-116, or hybridizes with one of these sequences under moderately stringent conditions. In

particular, sequences with the consecutive numbers 48 and 49 (in accordance with Table 1 ~~and sequence presentation in Fig-1~~) with a function as rRNA-methyl transferases (aviRa and aviRb) and also the sequences with the consecutive numbers 50 and 51 (in accordance with Table 1 ~~in combination with Fig-1~~) with a function as ABC transporter genes (aviABC1 and aviABC2), which impart resistance to avilamycins and/or sequences that correspond by at least 95% with these sequences with the aforementioned consecutive numbers, or hybridize with one of these sequences under moderately stringent conditions, are described in the present invention. For the remainder, even mixtures of nucleic acids that represent any subcombination of the nucleic acids, with the consecutive numbers 1 to 51 from Table 1, for example mixtures of two, three, four, ... , 50 nucleic acids in any combination, are also disclosed according to the invention, if applicable also as a combination on one nucleic acid strand or on different strands.

[0048] In this context, (a) nucleic acid(s) that correspond(s) by at least 95%, preferably 97%, and particularly precisely with the nucleic acid sequence in accordance with one of the sequences with the consecutive number 1 to 32 in accordance with Table 1 (~~in combination with Fig-1~~), preferably 1 to 7, particularly 1, 2, 4, or 6, or one of the sequences with the consecutive number 48 to 51 or 43, 44, or 46 in accordance with Table 1 (~~in combination with Fig-1~~) or that hybridize(s) with one of the sequences under moderately stringent conditions is/are particularly preferred.

[0049] Analogously, gene clusters that contain "Open reading frames," preferably 54, which correspond in their nucleic acid sequence by at least 95%, preferably 97%, and particularly precisely to the nucleic acid sequences according to the sequences with the consecutive numbers 1 to 54 (Table 1 ~~in combination with Fig-1~~) or hybridize with one of these sequences under moderately stringent conditions and that are arranged on a nucleic acid strand or in any combination on one of the other strands, preferably in accordance with Fig. 109 ~~1~~, are another object of the invention. The genes in a gene cluster according to the invention can contain 2, three, four, ... , 54 genes according to the invention, in any strand

distribution and subcombination, if applicable in combination with the genes already known, and in particular, the segments located between the ORFs can be any nucleotide sequence.

[0050] This particularly relates to a gene cluster in accordance with Fig. 1, but also to gene clusters that contain corresponding nucleic acids, possibly also in another arrangement, where it is preferred, but not necessary, that all the ORFs can be found in the gene cluster in accordance with the consecutive numbers 1-54 (Table 1 ~~in combination with Fig. 1~~).

Please replace paragraphs [0052] and [0053] with the following paragraphs:

[0052] It was possible to derive protein and polypeptide sequences from the newly discovered sequences of the ORFs or genes. Accordingly, another object of the invention is a protein or polypeptide that corresponds by at least 95%, preferably 97%, or particularly precisely to the amino acid sequence in accordance with one of the sequences with the consecutive numbers 55-101 (Table 1 ~~in combination with Fig. 1~~).

[0053] In this context, it is preferred if the protein or polypeptide according to the invention corresponds by at least 95%, preferably 97%, and particularly precisely to the amino acid sequence in accordance with one of the sequences with the consecutive number 55 to 86 or 97, 98 or 100 or 102 to 105 (Table 1 ~~in combination with Fig. 1~~), preferably 55 to 61, particularly 55, 56, 58 or 60.

Please replace paragraphs [0058] and [0059] with the following paragraphs:

[0058] Likewise, a cell that contains at least one nucleic acid modified by gene technology, the sequence of which, before modification, corresponded to the nucleic acid sequence in accordance with one of the sequences with the consecutive number 1 to 54 (Table 1 ~~in combination with Fig. 1~~) by at least 95%, preferably 97%, and particularly precisely that hybridized with one of these sequences under moderately stringent conditions, is also an object of the invention.

[0059] An especially preferred object of the invention is a cell of the type *Streptomyces viridochromogenes*, preferably of the subtype Tu57, in which at least one of the nucleic acids with a sequence with one of the consecutive numbers 1-54 (Table 1 ~~in combination with Fig. 1~~) was modified by gene technology or deleted. In this context, it is especially preferred if at least one of the nucleic acids with a sequence with the consecutive number 1 or 2-7 (Table 1 ~~in combination with Fig. 1~~) preferably with one of the sequences with the consecutive number 1, 2, 4, or 6 (Table 1 ~~in combination with Fig. 1~~), particularly with a sequence with the consecutive number 2 or with a sequence with consecutive numbers 2 and 1, 2 and 4, or 2 and 6 (in accordance with Table 1 ~~in combination with Fig. 1~~) was modified by gene technology or deleted.

Please replace paragraph [0063] with the following paragraph:

[0063] It is preferred if, in this process, the cell that can be cultivated is selected from a cell of the type *Streptomyces viridochromogenes* or a cell that, with the exception of the nucleic acid modified by gene technology, deleted or not expressed contains the nucleic acids in accordance with consecutive number 1-54 in accordance with Table 1 ~~in combination with Fig. 1~~, or nucleic acids homologous to them by at least 95%, preferably 97%, or sequences that hybridize with these sequences, or the gene cluster according to the invention. In the technical literature, the latter is referred to as heterologous expression. In this context, it is especially preferred if the cell is selected from a cell of the type *Streptomyces viridochromogenes*, *Streptomyces lividans*, *Streptomyces albus* or *Streptomyces fradiae*, especially a cell of the type *Streptomyces viridochromogenes* Tu 57 or A23575.

Please replace paragraph [0065] with the following paragraph:

[0065] It is furthermore preferred if, in the process according to the invention, the modified nucleic acid(s) coded for a methyl transferase and/or a halogenase. In this context, it is especially preferred if the sequence(s) of the modified nucleic acid(s) before modification correspond(s) by at least 95%, preferably 97%, and particularly precisely to the nucleic acid sequence(s) of at least one of the sequences with the consecutive numbers 1 or 2-7 in

accordance with Table 1 ~~in combination with Fig. 1~~, preferably one of the sequence with the consecutive number 2 or the sequences with the consecutive numbers 2 and 1, 2 and 4, or 2 and 6 (in accordance with Table 1 ~~in combination with Fig. 1~~).

Pease delete paragraphs [0077] through [0079].

Please replace paragraphs [0081] through [0085] with the following paragraphs:

[0081] Figure ~~109-1~~ shows the relative arrangement of the ORFs found on the gene cluster.

[0082] ~~Figure 110~~ Figures 2A-D show the mass spectrum of the products of the mutant *S. viridochromogenes* GW4.

[0083] Figure ~~111-3~~ shows the mass spectrum of the hydrolyzed products of the mutant *S. viridochromogenes* GW4.

[0084] Figure ~~112-4~~ shows a Southern blot with the mutant *S. viridochromogenes* GW4.

[0085] The assignment of the ORF abbreviation to its function and sequence (including the derived protein sequence) can be found in the following Table:

Table 1

Gene (ORF)/ protein or polypeptide	Function	Consecutive number: Gene (ORF) / protein or polypeptide in Figure 1	<u>SEQ ID NO.</u> <u>Nucleotide/</u> <u>amino acid</u>
AviX1	Regulation	8/62	<u>11/12</u>
AviX2		33/87	<u>13/14</u>
AviX3		34/88	<u>15/16</u>
AviX4		35/89	<u>17/18</u>
AviX5		36/90	<u>19/20</u>
AviRb	Resistance/methylation of the rRNA	48/102	<u>21/22</u>

Gene (ORF)/ protein or polypeptide	Function	Consecutive number: Gene (ORF) / protein or polypeptide in Figure 1	SEQ ID NO. Nucleotide/ amino acid
AviX6		37/91	<u>23/24</u>
AviX7		38/92	<u>25/26</u>
AviX8		39/93	<u>27/28</u>
AviRa	Resistance/methylation of the rRNA	49/103	<u>29/30</u>
AviQ1	Sugar biosynthesis	9/63	<u>31/32</u>
AviGT2	Biosynthesis of the heptasaccharide chain	10/64	<u>33/34</u>
AviX9		40/94	<u>35/36</u>
AviC1	Regulation	11/65	<u>37/38</u>
AviC2	Regulation	12/66	<u>39/40</u>
AviX10		41/95	<u>41/42</u>
AviX11		42/96	<u>43/44</u>
AviG1	Sugar biosynthesis (2-deoxy-D-avalose) / modification (methylation)	3/57	<u>45/46</u>
AviJ	Antibiotic transport	13/67	<u>47/48</u>
AviN	Biosynthesis of orsellinic acid	14/68	<u>49/50</u>
AviM	Biosynthesis of orsellinic acid	52/106	<u>51/52</u>
AviD	Sugar biosynthesis (D-olivose, 2-deoxy-D-avalose)	53/107	<u>53/54</u>
AviE1	Sugar biosynthesis (D-olivose, 2-deoxy-D-avalose)	54/108	<u>55/56</u>
AviQ2	Sugar biosynthesis	15/69	<u>57/58</u>
AviG5	Modification (methylation)	6/60	<u>59/60</u>
AviO1	Modification	43/97	<u>61/62</u>
AviGT1	Biosynthesis of the heptasaccharide chain	16/70	<u>63/64</u>
AviE2	Sugar biosynthesis	17/71	<u>65/66</u>
AviG2	Modification (methylation)	4/58	<u>67/68</u>
AviZ1	Sugar biosynthesis	18/72	<u>69/70</u>

Gene (ORF)/ protein or polypeptide	Function	Consecutive number: Gene (ORF) / protein or polypeptide in Figure 1	SEQ ID NO. Nucleotide/ amino acid
AviG6	Modification (methylation)	7/61	<u>71/72</u>
AviO3	Modification	44/98	<u>73/74</u>
AviG3	Modification (methylation)	5/59	<u>75/76</u>
AviX12		45/99	<u>77/78</u>
AviABC1	Antibiotic transport	50/104	<u>79/80</u>
AviABC2	Antibiotic transport	51/105	<u>81/82</u>
AviB1	Modification	19/73	<u>83/84</u>
AviB2	Modification	20/74	<u>85/86</u>
AviGT3	Biosynthesis of the heptasaccharide chain	21/75	<u>87/88</u>
AviGT4	Biosynthesis of the heptasaccharide chain	22/76	<u>89/90</u>
AviO2	Modification	46/100	<u>91/92</u>
AviP1	Sugar biosynthesis (L-lyxose)	23/77	<u>93/94</u>
AviQ3	Sugar biosynthesis	24/78	<u>95/96</u>
AviH	Modification (halogenation)	1/55	<u>97/98</u>
AviX13		47/101	<u>99/100</u>
AviG4	Modification (methylation)	2/56	<u>101/102</u>
AviE3	Sugar biosynthesis (4-O-methyl-D-fucose)	25/79	<u>103/104</u>
AviS	Sugar biosynthesis (D-olivose, 2-deoxy-D-evalose)	26/80	<u>105/106</u>
AviT	Sugar biosynthesis (D-olivose, 2-deoxy-D-evalose)	27/81	<u>107/108</u>
AviZ3	Sugar biosynthesis (D-olivose, 2-deoxy-D-evalose)	28/82	<u>117/118</u>
AviZ2	Sugar biosynthesis	29/83	<u>109/110</u>
AviX14	Regulation	30/84	<u>111/112</u>
AviX15	Regulation	31/85	<u>113/114</u>
AviX16	Regulation	32/86	<u>115/116</u>

Please replace paragraph [0087] with the following paragraph:

[0087] Standard methods of molecular biology, which are known to persons skilled in the art, were carried out. The isolation of *E. coli* plasmid DNA, DNA restriction, DNA modification as well as “filling in sticky ends” and “Southern” hybridization were carried out in accordance with the protocols of the manufacturers of the kits, enzymes, and reagents (Amersham-Pharmacia Boehringer Mannheim, Promega, Stratagene). *Streptomyces* protoplast formation, transformation, and regeneration were carried out in the usual manner. The PCR was carried out with a Perkin Elmer GeneAmp 2400 thermal cycler, where the conditions were as described and as usual. The oligonucleotide primers used were:

AviG4F (5'-GGACGCCTATCTGTGCCACCCCTTCCTGGT-3') SEQ ID NO.: 119

AviG4R (5'-TGAGCGCTCGCCTAGACAGAATCATCTCCC-3') SEQ ID NO.: 120

S2A (5'-GCGTCCATCTTGCCGGGA-3') SEQ ID NO.: 121

S2B (5'-CGTGGATCCCGCCGGCCC-3') SEQ ID NO.: 122

Also enclosed herewith is a copy of the sequence listing in CD-ROM format as file 1974005-SEQ-ENG, created on April 9, 2003 and comprising 2.54 MB to be entered as part of the present application.

Favorable consideration of this application as amended is respectfully requested.

Respectfully submitted,

A handwritten signature in cursive script, reading "Kathy Smith Dias", written over a horizontal line.

Kathy Smith Dias
Attorney for Applicants
Registration No.: 41,707

Dated: April 10, 2003.

HESLIN ROTHENBERG FARLEY & MESITI P.C.
5 Columbia Circle
Albany, New York 12203-5160
Telephone: (518) 452-5600
Facsimile: (518) 452-5579